Discovery of PRT3789, a first-in-class potent and selective SMARCA2 degrader in clinical trials for the treatment of patients with SMARCA4 mutated cancers

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Prelude THERAPEUTICS

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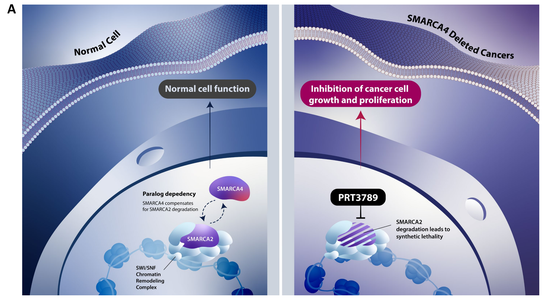
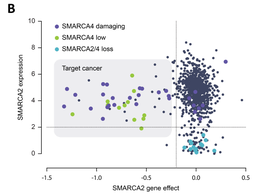
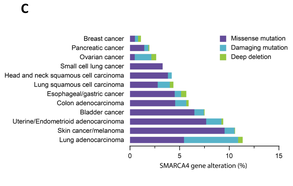
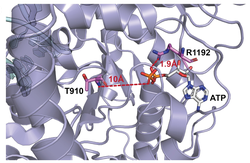
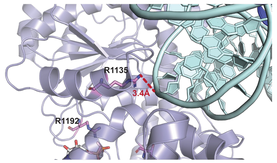
Background











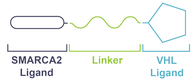
R1192 and **T910** are in close proximity to ATP bound to SMARCA4 and **likely impact ATP binding**

R1135 is in close proximity to DNA bound to SMARCA4 **and likely impacts DNA binding**

(A) SMARCA2 and SMARCA4 are the core catalytic subunits of the SWI/SNF complexes, which play an important role in controlling gene expression by remodeling chromatin. SMARCA4 is mutated in multiple cancers and SMARCA4-deficient cancer cells can become highly dependent on SMARCA2 for their survival. Therefore, targeting SMARCA2 in SMARCA4-deleted cancers using selective SMARCA2 degraders induces synthetic lethality, while sparing SMARCA4 wild-type normal cells; **(B)** Cell lines with SMARCA4 damaging mutation or low expression show high SMARCA2 gene dependency scores, suggesting the synthetic lethal relationship of targeting SMARCA2 and SMARCA4-deficiency; **(C)** Percentage of SMARCA4 mutations in different types of cancer. Datasets are from mixed studies (data extracted from cBioPortal in August 2021); **(D)** In addition to damaging mutation ("loss of SMARCA4 protein"), some tumors express SMARCA4 hotspot missense mutations near its ATP binding site or DNA binding site that likely alters biological function of SMARCA4. The sensitivity of cancer cells expressing such mutations to PRT3789 is under investigation.

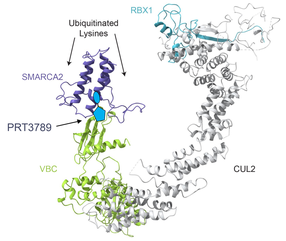
Discovery of PRT3789, a Selective SMARCA2 Degrader

Figure 2. Characterization of PRT3789



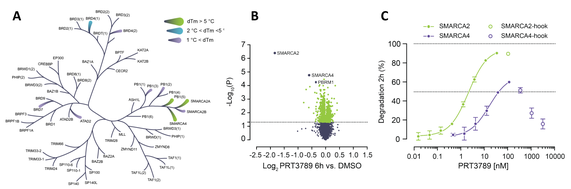
PRT3789, a VHL-based SMARCA2 degrader IV drug candidate

- Highly potent for SMARCA2 degradation in various cancer cell lines
- Highly selective for SMARCA2 over SMARCA4
  - Cellular assays: >1000× selective
  - HiBiT DC_{50}: ~40× selective
  - Selectivity confirmed *in vivo* PD
- Efficacious in SMARCA4 mutated CDX and PDX *in vivo* models at well-tolerated doses
- Clean on hERG and Safety 47
- Moderate CYP3A4 inhibition (IC_{50} >10-fold expected C_{max}), with no TDI



A 3D structure of SMARCA2 bromodomain and VHL-Elongin B/C, CUL2 and RBX1 complex. Ubiquitinated lysine residues were detected by Ubiquitin MS proteomics study.

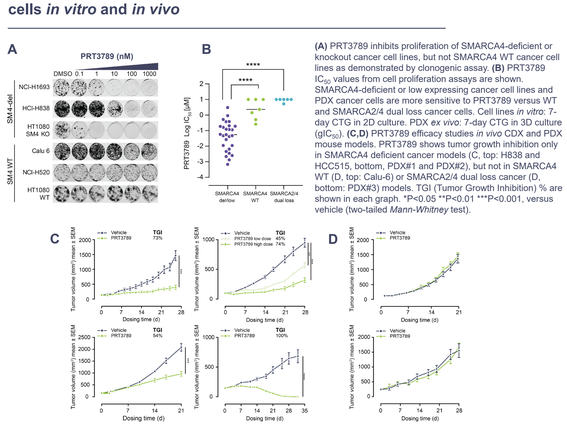
Figure 3. PRT3789 displays excellent SMARCA2 degradation selectivity and potency



Assay	PRT3789
Selectivity SM4/SM2 (HiBiT)	40X
SMARCA2 ICW DC_{50} (nM)	0.10 ~ 2.0
SMARCA4 ICW DC_{50} (nM)	> 1000

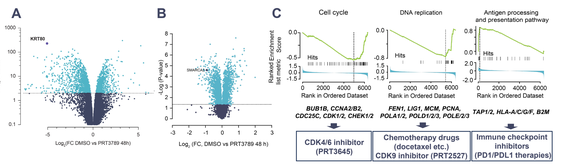
(A) DSF shows that PRT3789 is selective for bromodomain subfamily VIII. **(B)** Global proteomics shows PRT3789 to be highly selective against SMARCA2. **(C)** SMARCA2 and SMARCA4 HiBiT assays show potent SMARCA2 degradation and selectivity over SMARCA4. **(D)** SMARCA2 degradation in cancer cell lines analyzed by In-cell Western (ICW). **(E)** Summary of SMARCA2 degradation selectivity and potency. **(F)** SMARCA2 and SMARCA4 Western blot with lysates from Calu-6 cells treated with PRT3789.

Figure 4. PRT3789 selectively inhibits growth of SMARCA4 deficient cancer cells *in vitro* and *in vivo*



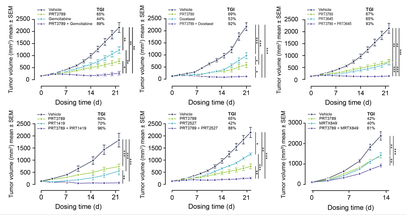
(A) PRT3789 inhibits proliferation of SMARCA4-deficient or knockout cancer cell lines, but not SMARCA4 WT cancer cell lines as demonstrated by clonogenic assay. **(B)** PRT3789 IC_{50} values from cell proliferation assays are shown. SMARCA4-deficient or low expressing cancer cell lines and PDX cancer cells are more sensitive to PRT3789 versus WT and SMARCA2/4 dual loss cancer cells. Cell lines *in vitro*: 7-day CTG in 2D culture. PDX *ex vivo*: 7-day CTG in 3D culture (gIC_{50}). **(C,D)** PRT3789 efficacy studies *in vivo* CDX and PDX mouse models. PRT3789 shows tumor growth inhibition only in SMARCA4 deficient cancer models (C, top: H838 and HCC515, bottom, PDX#1 and PDX#2), but not in SMARCA4 WT (D, top: Calu-6) or SMARCA2/4 dual loss cancer (D, bottom: PDX#3) models. TGI (Tumor Growth Inhibition) % are shown in each graph. *P<0.05 **P<0.01 ***P<0.001, versus vehicle (two-tailed *Mann-Whitney* test).

Figure 5. PRT3789 epigenetically regulates gene signatures in SMARCA4 deficient cancer cells, resulting in potential combination strategies



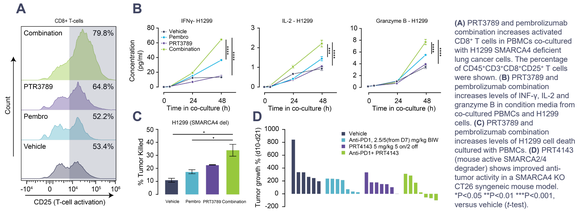
PRT3789 alters global transcriptome **(A)** or proteome **(B)** in SMARCA4 deficient cancer cells. Volcano plots display Log_2 (FC vs. DMSO) gene expression or protein expression in SMARCA4 deficient NCI-H1693 cells treated with PRT3789 for 48h. **(C)** Gene or protein set enrichment analyses show downregulated DNA replication and cell cycle pathway and upregulated antigen processing and presentation pathway. Biology MoA-based potential combination therapy partners are shown.

Figure 6. PRT3789 combination effects with FDA approved or investigational drugs



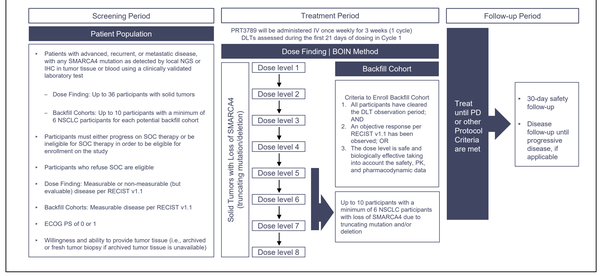
Combination of PRT3789 with indicated FDA approved SOC therapies (gemcitabine, docetaxel or MRTX849: KRAS (G12Ci) or investigational drugs (PRT3645: CDK4/6i, PRT1419: MCL1i, PRT2527: CDK9i) were tested *in vivo* appropriate CDX or PDX models. TGI (Tumor Growth Inhibition) % are shown in each graph. *P<0.05 **P<0.01 ***P<0.001, versus vehicle (two-tailed *Mann-Whitney* test).

Figure 7. Combination of PRT3789 with pembrolizumab may improve immune response against SMARCA4 deficient cancers



(A) PRT3789 and pembrolizumab combination increases activated $CD8^+$ T cells in PBMCs co-cultured with H1299 SMARCA4 deficient lung cancer cells. The percentage of $CD45^+CD3^+CD8^+CD25^+$ T cells were shown. **(B)** PRT3789 and pembrolizumab combination increases levels of INF-γ, IL-2 and granzyme B in condition media from co-cultured PBMCs and H1299 cells. **(C)** PRT3789 and pembrolizumab combination increases levels of H1299 cell death cultured with PBMCs. **(D)** PRT4143 (mouse active SMARCA2/4 degrader) shows improved anti-tumor activity in a SMARCA4 KO CT26 syngeneic mouse model. *P<0.05 **P<0.01 ***P<0.001, versus vehicle (*t*-test).

Figure 8. PRT3789-01 Phase 1 Study Design (NCT05639751)



Conclusions

- PRT3789 is a first-in-human potent and selective SMARCA2 degrader
- PRT3789 induces strong synthetic lethality in pre-clinical models of SMARCA4 mutated cancers
- PRT3789 is efficacious *in vivo* at well-tolerated doses in pre-clinical mouse models
- Evidence-driven potential combination therapies were explored in pre-clinical mouse models
- Currently enrolling patients with SMARCA4 mutated solid tumors in a Phase I dose escalation study in the United States and Europe (NCT05639751)

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Acknowledgments

We thank all the patients and their families in our ongoing clinical trials. The following CROs/Institutions provided data for this study: CrownBio and HD Biosciences (*in vivo* data); Genewiz (RNA-seq and ATAC-seq); The Wistar institute (Global Proteomics). Editorial support was provided by Arne Fabritius, Endosymbiont GmbH and was funded by Prelude Therapeutics, Inc.

Disclosures

1.Authors are or were employees of Prelude Therapeutics, Inc. at the time of research and may own equity in the Company.